claim 44, stand rejected as unpatentable over de Macario in view of Davis and further in view of Böcker.

Claim 1 has been amended both as to the claimed density of through-holes and to insert a space, inadvertently omitted, between the words 'second' and 'sample.'

Rejections based on 35 U.S.C. 112

The Recited Through-Hole Density

The rejection on grounds that the application lacks antecedent basis for claiming a "density of at least 1 through-holes per square millimeter" should not be applied to claim 1 since that language did not occur in the earlier version of the claim, and since claim 1, as now amended, requires a density of at least 1.6 through-holes per square millimeter. Nor does the offending limitation appear in any of the claims (3, 5-13) dependent from claim 1, so they, too, should not be subject to this rejection, the withdrawal of which is requested.

With respect to claims 14 and 41, each has been amended, in accordance with the Examiner's discussion, to claim a minimum density of 1.6 holes per square millimeter, (i.e., the square of the reciprocal of the hole-to-hole spacing of 800 μm, given, as an example of order of magnitude, in the first complete paragraph of page 6 of the application). Please withdraw this rejection with respect to amended claims 14 and 41, and dependent claim 15.

Indefinite Limitations

Claim 14 has been amended to remove recitation of a redundant step, and claim 17 has been amended for consistency with the plural number of through-holes in an antecedent claim. Applicant's representative appreciates the Examiner having brought these matters to our attention.

Rejections over Prior Art- De Macario ('890) in view of Davis and Böcker

De Macario's device cannot accommodate resolution of holes on the scale required in claims 1, 14, 16, and 41.

Each of the independent claims (1, 14, 16, and 41) stands rejected over the combination of de Macario ('890, hereinafter, "de Macario") and Davis (with Böcker further applied against claim 16).

Succinctly stated, de Macario teaches a plate for supporting fluid samples in a standard transmission spectrometer to replace conventional sample cuvettes. The de Macario sample holder is inserted, in one-for-one replacement of a cuvette holder, into a spectrometer, providing, according to de Macario's teachings, advantages such as lower fluid volume requirements per sample. The de Macario device, and its purpose, remain an "[apparatus] for use in a horizontal beam spectrophotometer is place of a conventional cuvette support that normally is used with said spectrophotometer." (de Macario, claim 1) There are inherent features of a device serving de Macario's purpose that are incompatible with the teachings, and especially with the claims, of the present invention.

De Macario cannot stand for the general use of holes to support liquid samples by surface tension. Such use of holes is notoriously well-known, such as for rings to hold bubbles in children's toys, or as inherent in a kitchen colander or strainer when wetted.

Instead, de Macario teaches a holder for liquid samples the absorptance (or reflectance, see de Macario, col. 5, lines 53 *ff*) of which are measured by moving the samples successively into the beam path of a spectrometer. The sample holder of de Macario must be interchangeable with a standard cuvette support receptacle in order to fulfill its function.

The sample holes taught in connection with applying the method of the present application (e.g., p. 6, line 4, where holes sizes of 100-400 μm are given as an example of order of magnitude) are smaller than the holes of de Macario (3 mm- col. 8, line 26) by an order of magnitude. But the reason for patentability of the present invention as claimed does *not* hinge on hole size.

Rather, the claimed method uses an extraordinary density of holes to provide surprising and useful results nowhere suggested by de Macario.

Reading de Macario most generously, it might be possible to achieve a density of de Macario holes (3-mm diameter) of, perhaps, 9 per square centimeter. Compare this with the density required in order to meet the limitation, present in claims 1, 14, 16, and 41, of **1600** per square centimeter! This difference is not one of size (to which the cited

case law refers) but absolute difference in quality of the physical phenomena required to implement the technology.

The claimed density is such that it is impossible, using any method suggest by de Macario, to spatially resolve the contents of each through-hole in a standard spectrometer, and, therefore, the teaching by de Macario that the plate sample holder be interchangeable with a standard cuvette holder *teaches away* from implementation of a density of through-holes such as taught by the present inventor.

The reason is one of 'depth of focus'. The source of a standard spectrometer (and its associated focusing optics) must be sufficiently far from the detector (and its associated collection optics) to accommodate the interposition of a sample. How much room is required? De Macario teaches that the 'width' dimension of the sample holder is 19 mm, i.e., somewhat less than an inch. Thus, the beam must be constrained to the size of the through-hole over a region of that distance in order to allow the sample to be interposed. The beam, in the case of the present invention, must 'fit' (taking the Airy disk as a criterion for crosstalk between channels) into a channel of ~100 μm diameter, and provide for imaging through that channel over a distance of 19 mm, if the beam is to sample a specified liquid (distinct from its neighbors, as taught and claimed in the present application).

Even allowing for optimal confocal focusing (as taught in the present application at p. 8, line 30), the diffraction-limited spread of a beam focused to 50 μm diameter (half the diameter of the through-hole) at the center of the source-to-detector space, after spreading out again over half the source-to-detector optics displacement of $L = 19/2 = 9.5$ mm (a minimum, based on de Macario) would be $50 + 1.22\,\lambda/D \cdot L = 50 + 1.22\,(.8/50) \cdot (9500\ \mu m) = 195\ \mu m$.[1] This is to say that a spectrometer beam that illuminates one hole will necessarily spill over into the position of a beam characterizing a neighboring hole before reaching the detector collecting optics.

Thus, de Macario *cannot* be taken to suggest sample densities anywhere near those required by the methods claimed in the present invention, because de Macario uses conventional spectrometer optics suited to measurements based on standard cuvettes.

[1] Derivation of the diameter of the Airy disk due to diffraction is provided in L. Levi, Applied Optics: A Guide to System Design, (Wiley, 1968), relevant pages of which are appended hereto.

Davis cannot be applied in combination with de Macario's for two reasons:

The de Macario system cannot accommodate a high density of through holes.

There is no suggestion in Davis that multiple fluids might, or could, populate adjacent holes.

As discussed above, the teachings of de Macario are limited by virtue of the requirement of interchangeability of the sample holder with a standard cuvette holder, to densities which do not include those claimed. Thus, there can be no suggestion in de Macario to combine sample holding with densities that exceed those described in de Macario by several orders of magnitude. For this reason alone, there is no suggestion to combine Davis with de Macario, other than after a person of ordinary skill in the instrumentation art has read the teachings of the present invention.

Moreover, Davis is inapposite for another reason that has been discussed in applicant's prior responses; Davis is drawn, very explicitly, to the proposition that a single liquid is applied, by dipping, to every one of the interstitial spaces of the Davis mesh. There is no reference in Davis to a plurality of liquids. It is not obvious (or even apparent, given that there is no discussion of the thickness of the mess material) how one skilled in the art might fill the Davis mesh with different samples, so there is certainly no suggestion to a person of ordinary skill in the art to do so. Therefore, a person of ordinary skill in the art would have no reason to combine the Davis mesh with the distinct sample technology of de Macario, until after studying the teachings of the present invention. Nor is the novel teaching allowing the recited densities of distinct samples provided by Böcker.

For these reasons, claim 1 (and dependent claims 3-13), claim 14 (and dependent claim 15), claim 16 and dependent claim 17, and claims 41 and 44 are all patentable over the references of record, separately or in combination.

Examination and allowance of the claims as amended is requested.

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For: METHOD FOR PERFORMING MICROASSAYS

MARKED UP CLAIMS TO SHOW AMENDMENTS

1. (amended three times) A method for analyzing specified properties of a set of samples, the method comprising:

a. providing a platen having two substantially parallel planar surfaces, an inner layer of hydrophilic material, two outer layers of hydrophobic material coupled to opposite sides of the inner layer, and a two-dimensional array of a plurality of addressable through-holes, the through-holes being disposed substantially perpendicularly to the planar surfaces <u>and the array characterized by an areal density of at least 1.6 through-holes per square millimeter</u>;

b. loading a first sample into a first set of through-holes of the two-dimensional array, the first sample being a liquid;

c. retaining the first sample in the first set of through-holes by surface tension;

d. adding a second sample into a specified through-hole, the specified through-hole having at least one adjacent through-hole containing a sample other than the second sample, the specified through-hole further coinciding with one of the first set of at least one of the though-holes thereby permitting a reaction between the first sample and the second_sample; and

e. characterizing the reaction in the through-hole in terms of the specified properties.

14. (amended three times) A method for characterizing a plurality of samples of distinct composition, the method comprising:

a. providing a platen having a set of through-holes comprising a two-dimensional array with a density of at least [one] 1.6 through-holes per square millimeter;

b. loading a specified sample into each through-hole of a first subset of the set of through-holes;

c. [loading a specified sample into each through-hole of a first subset of the set of through-holes; d.] loading a second sample into at least one through-hole adjacent to a hole of the first subset of through-holes in such a manner as to substantially prevent capillary outmigration of the second sample; and [e.]

d. characterizing a property of the specified sample.

16. (amended three times) A method for analyzing a plurality of samples, the system comprising:

a. loading the samples into a plurality of through-holes disposed in a platen in a two-dimensional array characterized by an areal density of at least 1.6 through-holes per square millimeter;

b. illuminating a set of more than one of the plurality of through-holes with optical radiation; and

c. separately analyzing the optical radiation emanating from each through-hole of the set of more through-holes than one using an optical arrangement including a detector array.

17. (amended) A method in accordance with claim 16, wherein the step of analyzing includes spectrally characterizing the optical radiation emanating from the [at least one] plurality of through-holes.

41. (amended) A method for characterizing a plurality of samples, the method comprising:

a. providing a platen having a two-dimensional array of through-holes;

b. loading a specified sample into each through-hole of a subset of the set of through-holes with a density of at least [one] 1.6 through-holes per square millimeter; and

c. characterizing a property of the specified sample.

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Applied Optics

A Guide to Optical System Design/Volume 1

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$$\beta = \frac{kb}{f} = \frac{2\pi b}{\lambda f} \tag{2.156b}$$

and we have written f in place of the clumsier r_0'.

This pattern (2.155) consists of a rectangular grid of dark lines at

$$x = \frac{n\lambda f}{2a}, \tag{2.157a}$$

$$y = \frac{n\lambda f}{2b}, \qquad n = 1, 2, 3, \ldots, \tag{2.157b}$$

with a "hill" of light in each of the rectangles cut out by this grid.

The location of the maxima and their height relative to that of the central maximum ($x = y = 0$) can be obtained from the data of Table 16, which lists the maxima of $(\sin x/x)^2$.

On integrating the illumination and equating it to the total power (P) passing the rectangular aperture, we find

$$M_0 = \frac{PA}{\lambda^2 f^2}, \tag{2.158}$$

where

$$A = 4ab.$$

is the area of the aperture and we have made use of the definite integral

$$\int_{-\infty}^{\infty} \left(\frac{\sin x}{x}\right)^2 dx = \pi.$$

When considering a long slit, one factor of (2.155) approaches a delta-function and the pattern has the form

$$M = \left(\frac{\sin \alpha x}{\alpha x}\right)^2 \quad \text{at} \quad y = 0 \tag{2.159}$$

$$= 0, \text{ elsewhere.}$$

A plot of (2.159) is shown in Figure 2.15a.

2.3.2.2 Circular Apertures. Turning to the circular aperture, radius a, we perform the integration of (2.139a) in polar coordinates and substitute

$$X = R\cos\theta, \qquad Y = R\sin\theta, \tag{2.160}$$

$$x = r\cos\varphi, \qquad y = r\sin\varphi.$$

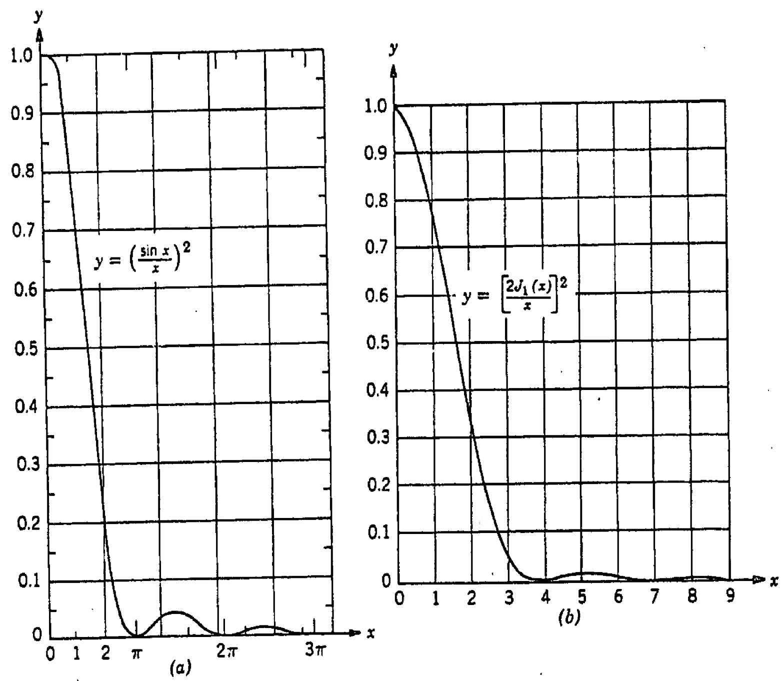

Figure 2.15 (*a*) Fraunhofer diffraction at a rectangle aperture: the function $y = (\sin x/x)^2$; (*b*) Fraunhofer diffraction at a circular aperture: the function $y = [2J_1(x)/x]^2$.

We choose the element of integration as $R\,dR\,d\theta$. The integral (139*a*) then becomes

$$U = c\int_0^a R\int_0^{2\pi} \exp\left[i\frac{kr}{f}(\cos\theta\cos\varphi + \sin\theta\sin\varphi)R\right] d\theta\, dR$$

$$= c\int_0^a R\int_0^{2\pi} \exp\left[\frac{ikrR}{f}\cos(\theta-\varphi)\right] d\theta\, dR. \qquad (2.161)$$

We note the well-known definite integral[14]

$$\int_0^{2\pi} e^{ix\cos\alpha}\,d\alpha = 2\pi J_0(x), \qquad (2.162)$$

where $J_0(x)$ is the zero-order Bessel function.

Because of the periodicity of the integrand, any finite value may be added to both limits of integration simultaneously, without affecting the

value of the integral. Hence the integral of (2.161) may be written

$$U = 2\pi c \int_0^a R J_0\left(\frac{krR}{f}\right) dR. \tag{2.163}$$

From the recurrence relation of Bessel functions

$$\frac{d}{dx}[x^{n+1}J_{n+1}(x)] = x^{n+1}J_n(x),$$

it follows that

$$\int x J_0(x)\, dx = x J_1(x), \tag{2.164}$$

so that the integral (2.163) may be written

$$U = (\pi a^2 c)\frac{2J_1(kra/f)}{(kra/f)}. \tag{2.165}$$

On squaring, we find that the intensity distribution is

$$\boxed{M = \left[\frac{2J_1(kra/f)}{kra/f}\right]^2 M_0,} \tag{2.166}$$

$$M_0 = \frac{AP}{\lambda^2 f^2}, \tag{2.158}$$

where A is again the aperture area, and we have made use of the fact that

$$J_1(x) \approx \frac{x}{2}, \qquad |x| \to 0.$$

A plot of (2.166) is shown in Figure 2.15*b*. The corresponding distribution is called an *Airy*[25] *pattern* and the region enclosed by the first minimum is called an *Airy disk*. From the data in Table 17 we find that the diameter of the Airy disk is

$$D_A = 2r \approx 1.22\frac{\lambda f}{a} \approx 1.22\frac{\lambda}{\sin\alpha'}, \tag{2.167}$$

where α' is the apex half-angle of the cone converging on the image point.

A number of maxima and minima of this function are tabulated in Table 17.

Integrating the illumination (2.166) over a region of radius r_1, we can show that the encircled power is

$$p(r_1) = \frac{P'(r_1)}{P} = 1 - J_0^2\left(\frac{kar_1}{f}\right) - J_1^2\left(\frac{kar_1}{f}\right). \tag{2.168}$$

[25]G. B. Airy (1835).

TABLE 16 MAXIMA OF $(\sin x/x)^2$
(Section 2.3.2.1)

x		$(\sin x/x)^2$
0		1.00000
4.49341	1.43030π	0.0471904
7.72525	2.45902π	0.0164800
10.9041	3.47088π	0.0083403
14.0662	4.47741π	0.0050287

TABLE 17 MAXIMA AND MINIMA OF $[2J_1(x)/x]^2$ (Section 2.3.2.2)

Dark ring No.	x		$J_1(x)^2$	$J_0^2(x)$	$[2J_1(x)/x]^2$
	0	0	0	1.00000	1.00000
1	3.83171	1.21967π	0	0.162216	0
	5.13562	1.63472π	0.115376	0	0.0174980
2	7.01559	2.23313π	0	0.090072	0
	8.41724	2.67929π	0.073647	0	0.0041579
3	10.17347	3.23832π	0	0.062350	0
	11.61984	3.69871π	0.054028	0	0.0016006
4	13.32369	4.24106π	0	0.047681	0
	14.79595	4.70970π	0.042659	0	0.0007794
5	16.47063	5.24276π	0	0.038600	0